CLAIM AMENDMENT

Please cancel claim 3. Please amend the claims as indicated below:

1. (Currently amended) A method of obtaining a bacterium comprising a nucleic acid sequence encoding a binding protein capable of binding a target ligand comprising the steps of:

(a) providing a Gram negative bacterium comprising a nucleic acid sequence encoding a candidate binding protein, wherein said binding protein is expressed in soluble form in the periplasm of said bacterium;

(b) contacting said bacterium with a labeled ligand capable of diffusing into said ~~bacterium~~ periplasm; and

(c) selecting said bacterium based on the presence of said labeled ligand within the periplasm~~bacterium~~, wherein said ligand and said candidate binding protein are bound in said bacterium.

2. (Original) The method of claim 1, further defined as a method of obtaining a nucleic acid sequence encoding a binding protein capable of binding a target ligand, the method further comprising the step of:

(d) cloning said nucleic acid sequence encoding said candidate binding protein.

3. (Canceled) The method of claim 1, wherein said binding protein is expressed in soluble form in the periplasm of said bacterium.

4. (Currently amended) The method of claim ~~3~~1, wherein said nucleic acid sequence encoding a candidate binding protein is further defined as operably linked to a leader sequence capable of directing expression of said candidate binding protein in said periplasm.

5. (Original) The method of claim 1, wherein said Gram negative bacterium is an *E. coli* bacterium.

6. (Original) The method of claim 1, further defined as comprising providing a population of Gram negative bacteria.

7. (Original) The method of claim 6, wherein said population of bacteria is further defined as collectively capable of expressing a plurality of candidate binding proteins.

8. (Original) The method of claim 7, wherein said population of bacteria is obtained by a method comprising the steps of:

(a) preparing a plurality DNA inserts which collectively encode a plurality of different potential binding proteins, and

(b) transforming a population of Gram negative bacteria with said DNA inserts.

9. (Original) The method of claim 6, wherein said population of Gram negative bacteria is contacted with said labeled ligand.

10. (Original) The method of claim 1, wherein said candidate binding protein is further defined as an antibody or fragment thereof.

11. (Original) The method of claim 1, wherein said candidate binding protein is further defined as a binding protein other than an antibody.

12. (Original) The method of claim 1, wherein said candidate binding protein is further defined as an enzyme.

13. (Original) The method of claim 1, wherein said candidate binding protein is further defined as not capable of diffusing out of said periplasm in intact bacteria.

14. (Original) The method of claim 1, wherein said labeled ligand comprises a peptide.

15. (Original) The method of claim 1, wherein said labeled ligand comprises a polypeptide.

16. (Original) The method of claim 1, wherein said labeled ligand comprises an enzyme.

17. (Original) The method of claim 1 where said labeled ligand comprises a nucleic acid.

18. (Original) The method of claim 1, wherein said labeled ligand is further defined as comprising a molecular weight of less than about 20,000 Da.

19. (Original) The method of claim 1, wherein said labeled ligand is further defined as comprising a molecular weight of less than about 5,000 Da.

20. (Original) The method of claim 1, wherein said labeled ligand is further defined as comprising a molecular weight of greater than 600 Da and less than about 30,000 Da.

21. (Original) The method of claim 1, wherein said labeled ligand is further defined as fluorescently labeled.

22. (Previously presented) The methods of claim 1, wherein said nucleic acid encoding a candidate binding protein is further defined as capable of being amplified following said selection.

23. (Original) The method of claim 1, further comprising treating said bacterium to facilitate said diffusing into said periplasm.

24. (Original) The method of claim 23, comprising treating the bacterium with hyperosmotic conditions.

25. (Original) The method of claim 23, comprising treating the bacterium with physical stress.

26. (Original) The method of claim 24, comprising treating the bacterium with a phage.

27. (Original) The method of claim 1, wherein said bacterium is grown at a sub-physiological temperature.

28. (Original) The method of claim 27, wherein said sub-physiological temperature is about 25°C

29. (Original) The method of claim 1, further comprising removing labeled ligand not bound to said candidate binding protein.

30. (Previously presented) The method of claim 1, wherein said selecting comprises fluorescent activated cell sorting.

31. (Original) The method of claim 1, wherein said selecting comprises magnetic separation.

32. (Original) The method of claim 1, wherein said ligand and said candidate binding protein are reversibly bound in said periplasm.

33-74. (Withdrawn)

RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 1-74 were initially filed. Claims 33-74 have been withdrawn from consideration as directed to non-elected subject matter. Claim 3 was canceled herein without prejudice or disclaimer. Support for the claim amendments is found in the claims as filed. Claims 1-2 and 4-32 are currently pending in the application and presented herein for reconsideration.

B. Rejections Under 35 U.S.C. §112, First Paragraph-Enablement

The Action maintains the rejection of claims 1-32 under 35 U.S.C. §112, first paragraph, as not being enabled. In particular, the Action presents three main points of alleged non-enablement: (1) it is stated that the claims are not enabled in the absence of a wash step, (2) it is stated that enablement is not provided for use of ligands greater than 2000 Da, and (3) it is alleged that use of the claimed method with a nucleic acid as the labeled ligand is not enabled. It is additionally stated that use of enzymes generally with the invention is not enabled. Applicants respectfully traverse as set forth below.

(1) Applicants have demonstrated that a wash step is not necessary

Applicants note that the addition of a wash step is not required for use of the invention as alleged in the Action. In support of this, Applicants have provided as **Exhibit A** the Declaration of Jongsik Gam. As described by Mr. Gam, the claimed methods have been used both with and without the addition of a wash step to demonstrate that the wash step is not necessary. It was demonstrated in particular that cells expressing a binding protein having affinity for a labeled ligand can be detected away from control cells based on the presence of the labeled ligand bound in the periplasm with or without a wash step. Mr. Gam explains that the specific interaction of

the binding protein and labeled ligand in the periplasm of the bacterium ***retains*** and ***concentrates*** the labeled ligand inside the periplasm of ***only*** those cells with high affinity binding proteins. The concentration of the labeled ligand bound to the binding protein in the periplasm makes the cells detectable regardless of the presence of unbound labeled ligand.

As Applicants have demonstrated that this additional step is not required, and the claimed method is fully enabled without the step, removal of the rejection is respectfully requested.

(2) The invention is not properly limited to ligands of less than 2000 Da or specific classes of ligands

The Action continues to reject the claims based on the allegation that labeled ligands of more than 2000 Da are not "capable of diffusing" into a cell. The evidence Applicants provided in the previous response included a peer reviewed publication by Chen *et al.*, (IDS ref. C54) showing that oligonucleotide 20mers (8,727 Da) enter the bacterial cell. However, the Action asserted that this was unpersuasive based on the allegation that facilitated transport is required for molecules of such size to enter the cell.

a. Interpretation of the claims

Applicants initially note that the maintenance of the rejection appears to be based on confusion regarding the meaning of the term "capable of diffusing" as used in claim 1. The Action has cited a definition from Webster's for the term "diffusion" which is said to be limited to spontaneous (*e.g.*, unfacilitated) movement. However, the claims are not so limited. The definition given is but one of several library definitions available for terms that are related to the actual original claim term "capable of diffusing into said bacterium." Neither the other definitions nor the specification are limited in this way.

The relevant definition of "diffuse," which has the inflected form "diffusing," from the Merrian-Webster™ Online dictionary is "to spread out or *become transmitted especially by contact.*" **Exhibit B** (emphasis added). Similarly, the Cambridge Online Dictionary™ (http://dictionary.cambridge.org/) defined "diffuse" as a verb to mean "1 to (cause something to) spread in many directions" or "(2) to (cause a gas or liquid to) *spread through or into a surrounding substance by mixing with it.*" **Exhibit C** (emphasis added).

Consistent with the foregoing, nothing in the specification requires that labeled ligands enter the bacterium by any given mechanism. Therefore, neither the art known meaning of the claim terms nor the specification are consistent with the position taken in the Action regarding the interpretation of "diffusing." Even taking the position as true for the purpose of this argument, although it is not, this at best goes to "how" or "why" the invention works, which is irrelevant under the first paragraph of 35 U.S.C. §112, first paragraph. All that is relevant under this section is that one of skill in the art can make and use the claimed invention without undue experimentation, which has fully been demonstrated herein and in Applicants previous responses.

b. Chen *et al.* demonstrates the enablement of the claims

Enablement speaks to the ability to make and use the claimed invention. The mechanisms by which ligands enter the cell is irrelevant to the function of the technique. Here, Applicants have submitted a peer-reviewed article, Chen *et al.*, that specifically shows that ligands of at least 10kDa can enter the periplasmic space. Regardless of the molecular mechanisms involved, this provides clear evidence for enablement of ligands of at least the 10 kDa acknowledged by the Action.

The Action states that the Chen *et al.* reference is not persuasive because "proper conditions" are required such as treatments with filamentous bacteriophages or growth under sub-optimal conditions. However, the specification ***specifically teaches*** these techniques as well as many other such techniques for permeabilization of bacteria. For example, the use of ***filamentous bacteriophages*** to increase permeability is described in the last paragraph of ***page 16*** of the specification. The use of ***sub-optimal temperatures*** is specifically taught in the first paragraph of ***Example 3*** of the specification. The specification further describes numerous other techniques that were known in the art for increasing bacterial permeability. For example, Fig. 8 shows that an increase in FACS signal was obtained when cells expressing periplasmic scFv antibodies to digoxigenin were labeled with this probe using 5X PBS to permeabilize the outer membrane. Therefore, given that the "proper conditions" for use of labeled ligands of at least 10 kDa are both fully described in the specification and known in the art, there is no basis whatsoever to conclude that Applicants' claims are not fully enabled.

c. The rejection was not applied with respect to the claimed invention

Applicants finally note that the rejection appears to have not been applied with respect to the claimed invention. All that is required under 35 U.S.C. §112, first paragraph, is that the specification teach one reasonably skilled in the art how to make and use ***what is claimed*** without undue experimentation. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988); *see also Durel Corp. v. Osram Sylvania Inc.*, 256 F.3d 1298, 1306-07 (Fed. Cir. 2001). The focus of examination must therefore be whether the subject matter within the scope of the claims is enabled. M.P.E.P. § 2164.08.

Here, step (b) of current claim 1 comprises contacting a bacterium with a labeled ligand "capable of diffusing" into the periplasm of the bacterium. To the extent that a given ligand is

not capable of diffusing into the periplasm as alleged by the Action, the ligand is not within the scope of the claims. Therefore, even assuming *arguendo* that the allegations in the Action are correct, which they are not, the full scope of the claims is enabled. *Id.*

In view of the foregoing, removal of the rejection is respectfully requested.

(3) The use of nucleic acids has been enabled

The previous evidence by Applicants found in the ***working examples*** demonstrating enablement for nucleic acids as ligands appears to have been held non-persuasive based on the issue discussed above regarding the meaning of "diffusing" and also because the claims do not recite the periplasm.

In response, Applicants note that the issue appears to be moot in light of the comments above demonstrating that the claims do not require that a labeled ligand enter the periplasm by any particular method and in view of the amendment to claim 1. Further, as was indicated previously, the Chen *et al.* (2001) reference describes the use of labeled ligands comprising nucleic acids for detection in the periplasm of *E. coli* bacteria expressing a binding protein. In particular, oligonucleotide 10mers (4,897 Da) and even 20mers (8,727 Da) were labeled with digoxigenin and a fluorescent label, resulting in the successful labeling of *E. coli* in 5X PBS expressing scFv antibodies binding the digoxin in the periplasm. (see Fig. 3, page 539). The studies demonstrate enablement for use of nucleic acids. Such evidence cannot be ignored.

Use of labeled ligands comprising nucleic acids is also described in Example 8 of the specification. This example demonstrates that a fluorescently-tagged oligonucleotide (2384 Da molecular weight) diffused into bacteria, and specific ligand-binding protein binding occurred. No significant DNA-labeled probe binding was observed in Example 8, and, because no evidence exists that this kind of interaction would present a significant problem for use of the

invention, the idea that DNA-labeled probe binding will prevent use of the invention must be regarded as speculation. The nucleic acids that are used as ligands can be easily distinguished from other nucleic acids because the ligand nucleic acids are tagged with a fluorescent (or other) labeling moiety.

The studies above demonstrate that nucleic acids can enter the periplasm and that entry of labeled ligands into the periplasm is not limited to only certain types of molecules of very limited size. In this case, the nucleic acid is not used as a source of genetic information to be transcribed and translated into protein. Rather, the nucleic acid is being used as a scaffold to contain the ligand of the binding protein as well as a fluorescent label. Therefore, again, non-specific binding with native nucleic acids does not affect the ability to identify selectively bound labeled ligand.

(4) Use of enzymes is enabled

The Action continues to assert that the specification does not enable use of enzymes generally. The previously submitted evidence was held non-persuasive based on the continued assertion that only certain types of molecules that are less than 900 Da can enter the bacterial cell. In response, Applicants note that the rejection should be moot in view of the evidence presented above illustrating that the mechanism by which ligands enter a cell is irrelevant and that molecules of at least 10 kDa can readily enter the periplasm. The rejection should further be moot because the claims require that the labeled ligand be capable of diffusing into the periplasm. The claims are thus fully commensurate with the scope of enablement.

The membrane permeabilization methods described by Applicants further are non-specific. This is supported by the multiple different types of labeled ligands that were used and shown to successfully enter the periplasm, including nucleic acids, oligopeptides and a

polyethylene glycol derivative, including substrate for *Fusarium solani* lipase cutinase. There is no basis to conclude that only certain types of substrates will cross the outer membrane.

No basis has also been provided to conclude why the *Fusarium solani* lipase cutinase example is not representative of the claims in general. This constitutes a working example within the scope of the claims. Given the demonstrated ability to introduce a wide variety of molecules of different sizes into the *E. coli* periplasm, there is no basis to conclude that the example does not enable the full scope of the claims. Any suggestions to the contrary are unsupported speculation given the evidence presented by Applicants.

In view of the foregoing, Applicants respectfully request the removal of the rejection of claims 1-32 under 35 U.S.C. § 112, first paragraph.

C. Rejections Under 35 U.S.C. §112, Second Paragraph

The Action rejects claims 1-32 under 35 U.S.C. §112, second paragraph, as being incomplete for the omission of a wash step. Applicants respectfully traverse.

As demonstrated in the Declaration of Mr. Gam as described herein above, the addition of a wash step is not required for the function of the invention and therefore this element need not be recited in the claims. Applicants need only recite those elements that are essential to the function of the invention. It is not required that non-essential embodiments from the disclosure be included in the claims. It is therefore respectfully submitted that the claims are fully definite and removal of the rejection is thus requested.